RAFF contrast during gene therapy of rat brain tumors: association with cell density in tissue undergoing apoptotic cell death

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Introduction: Cell death via apoptosis is the goal of a variety of cancer therapies, including hormone, chemo, irradiation and gene therapies, because it often leads to a favorable outcome [1]. Early detection of apoptosis and/or early assessment of therapeutic response is clinically important, particularly when individualizing treatment protocols. The BT4C rat glioma transfected with herpes simplex thymidine kinase (HSV-tk) gene has been proven to provide a good apoptotic cell death model [2] and it has been well characterized by several MRI markers, such like diffusion, $T_{1\rho}$ and proton spectroscopy [3,4]. In the current work we extended the recently developed method known as relaxation along fictitious field (RAFF) [5] and applied it to detect apoptotic cell death in BT4C HSV-tk gene therapy model. Associations between RAFF relaxation times with the trace of water diffusion tensor (Tr{D}) and histology derived cell density are also investigated.

Materials and Methods: 10^4 BT4C glioma cells, transfected with viral HSV-tk gene, were implanted to a depth 2.5 mm into the corpus callosum of female BDIX rats (180-250 g) in 5μL of Optimem. After 3-4 weeks, rats in the treatment group (n=11) were treated with ganciclovir (25 mg/kg *i.p.*) twice daily up to 8 days, while the rats without treatment (n=5) served as a control group. MRI experiments were carried out at 4.7 T with a Varian ^{UNITY}INOVA console and a quadrature half-volume surface coil. The Tr{D} was obtained in a single acquisition using four diffusion encoding directions in a double spin-echo sequence incorporating an adiabatic refocusing pulse with b-values 0, 527, and 915 s/mm^2. The idea to apply RAFF is to measure relaxation in a presence of fictitious field (**E**) during RF irradiation. The **E** was created by *sine* amplitude modulation $\omega_1(t) = \omega_1^{max} \sin(\omega_\alpha t)$ and *cosine* frequency modulation $\Delta\omega(t) = \omega_1^{max} \cos(\omega_\alpha t)$ functions (SC), where ω_1^{max} is the maximum amplitude of $\omega_1(t)$, leading to the condition $|d\alpha/dt| = \omega_\alpha$ = constant, $\alpha(t) = \tan^{-1}(\omega_1(t)/\Delta\omega(t))$. The amplitude of **E** is $E = (\omega_{eff}^2+(d\alpha/dt)^2)^{1/2}$ = constant during the SC pulse, where $\omega_{eff} = (\omega_1^2+\Delta\omega^2)^{1/2}$. For the contrast modifications, the angle ε is defined by the relation $\varepsilon = \tan^{-1}(\omega_\alpha/\omega_{eff})$. In the RAFF experiments $\omega_1^{max}/(2\pi) = 625$ Hz, ε = 30, 45, 60 and 83° (**Fig. 1**) and number of segments (2-64) leading to pulse train lengths of 4.53-144.82 ms. For comparison, rotating frame relaxations during adiabatic pulses were measured as described before [6,7] with $\omega_1^{max}/(2\pi) = 2.5$ kHz and $T_p = 2.26$ ms to match pulse train length with RAFF. For comparison with RAFF, conventional on-resonance $T_{1\rho}$ measurements were conducted using adiabatic spin-lock pulse [8] with matching the ω_1^{max} and continuous-wave pulse lengths with RAFF. Signal intensity decays were measured from a transverse slice in the plane including maximum area of tumor (all methods), using fast spin-echo imaging readout (TR/TE=2.5 s/ 64 ms, slice thickness = 1 mm) (RAFF and rotating frame relaxation methods). A mono-exponential decay curve was fitted into signal intensities with parameters S_0, and T_{RAFF} using a least square method in Matlab. Viable cells were counted from Nissl-stained sections using Stereo Investigator software in a NeuroLucidia morphometry system. Using Pearson correlation, correlation coefficients R and significance levels p were calculated for associations between measured parameters.

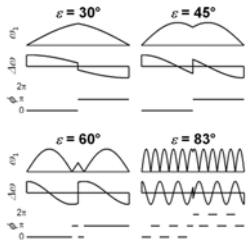

Fig. 1 *Amplitude $\omega_1(t)$ and frequency $\Delta\omega(t)$ modulation functions of RAFF pulse for used angles ε.*

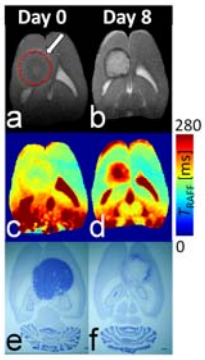

Fig. 2 *T_2 weighted transversal slices from tumor level at days 0 (a) and 8 (b) with corresponding RAFF (ε = 45°) maps (c,d). Nissl-stained sections from control (e) and treated (f) animals. An arrow and a dashed circle show tumor tissue in (a).*

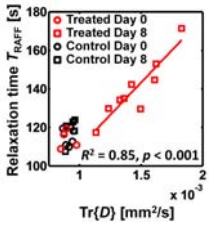

Fig. 3 *Correlation between RAFF (ε = 60°) relaxation times and water Tr{D}.*

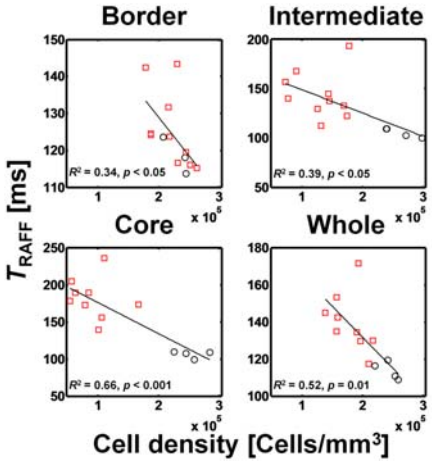

Fig. 4 *Correlation between RAFF (ε = 60°) relaxation times and cell densities in different areas of tumor. For markers see **Fig. 3**.*

Results: As a control parameter we measured tumor volumes, which increased until day 4 after and then decreased in the treated group, as seen from **Fig. 2ab**, and gradually increased during the whole 8 day period in control group. RAFF relaxation time (T_{RAFF}) maps show increase in the T_{RAFF} during the therapy in the whole tumor area (**Fig. 2cd**). Nissl-stained histological sections show the drop in cell density between the control (**Fig. 2e**) and treated tumor (**Fig. 2f**). Water Tr{D} increased during the gene therapy and associations between water Tr{D} and T_{RAFF} were found to be significant (R^2 = 0.68 - 0.85, p < 0.01) for all ε used at day 8 (**Fig. 3**). Significant associations between T_{RAFF} and rotating frame relaxation times were also found (data not shown). T_{RAFF} and cell counts were averaged separately in border, intermediate and core areas of the tumors, and associations were found to be significant in all areas for angles ε = 45 and 60° (**Fig. 4**).

Discussion: The current study shows that RAFF pulses can be applied with various ε angles *in vivo*. The water Tr{D} values and associations with cell density were highly consistent with previous findings [4]. The associations between T_{RAFF} with water Tr{D} show similar trend with continuous-wave spin-lock experiments as expected [4]. The association between T_{RAFF} and water Tr{D} might reflect the presence of diffusion driven relaxation pathway in RAFF. Within the investigated MR technique, RAFF with ε = 45 and 60° were the only methods which provided associations with cell density in all tumor areas, manifesting sensitivity of RAFF to small alterations in cell density. Therefore, potential applications for RAFF besides detection of cancer therapy may include other applications where the cell density is altered due to disease (for example, degeneration of dopaminergic neurons in *substantia nigra* during Parkinson's disease).

References: [1] Hakumäki, Liimatainen, EJR 56 (2005) 143-153, [2] Poptani et al., Cancer Gene Ther 5 (1998) 101–109, [3] Kauppinen, NMR biomed 15 (2002) 6-17, [4] Kettunen et al., Radiology 243 (2007) 796-803, [5] Liimatainen et al., Proc.Int. Soc.Magn.Res.Med. Toronto (2008) 639, [6] Michaeli et al., JMR 169 (2004) 293-299, [7] Michaeli et al., JMR 181 (2006) 135-147, [8] Gröhn et al., MRM 54 (2005) 14-19.

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